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1: Ma HH, Yao JL, Li G, Yao CL, Chen XJ, Yang SJ. Related Articles, Links

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2: Haack K, Cockrell AS, Ma H, Israeli D, Ho SN, McCown TJ, Kafri T. Related Articles, Links

Transactivator and structurally optimized inducible lentiviral vectors.
Mol Ther. 2004 Sep;10(3):585-96.
PMID: 15336658 [PubMed - in process]

3: Ma H, Kafri T. Related Articles, Links

A single-LTR HIV-1 vector optimized for functional genomics applications.
Mol Ther. 2004 Jul;10(1):139-49.
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In vivo gene delivery of urokinase-type plasminogen activator with regulatable lentivirus induces behavioural changes in chronic cocaine administration.
Eur J Neurosci. 2004 Dec;20(12):3473-88.
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Transactivator and structurally optimized inducible lentiviral vectors.
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5: Bahi A, Boyer F, Kafri T, Dreyer JL. Related Articles, Links

CD81-induced behavioural changes during chronic cocaine administration: in vivo gene delivery with regulatable lentivirus.
Eur J Neurosci. 2004 Mar;19(6):1621-33.
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Methods Mol Biol. 2004;246:367-90. Review.

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L11	49	S L8 AND SIN
L12	25	DUP REM L11 (24 DUPLICATES REMOVED)

IN Kafri, Tal; Ma, Hong
SO U.S. Pat. Appl. Publ., 23 pp.
CODEN: USXXCO
TI Single LTR lentivirus vector for generating high titer vector particles and optimized for functional genomics applications
AB The present invention provides lentivirus vectors comprising a single retroviral LTR, a polypurine tract, a packaging signal, a primer binding site and a rev responsive element. Further provided is a lentivirus vector comprising a heterologous nucleotide sequence, a single retroviral long terminal repeat (LTR), a packaging signal, a rev responsive element, a polypurine tract, a eukaryotic promoter, a primer binding site, a bacterial origin of replication and a bacterial selection marker. In addn., the present invention provides an isolated nucleic acid comprising a 5' retroviral LTR and a 3' retroviral LTR, a heterologous nucleotide sequence, a packaging signal, a rev responsive element, a polypurine tract, a eukaryotic promoter, a primer binding site, a bacterial origin of replication and a bacterial selection marker cassette, wherein the bacterial origin of replication and bacterial selection marker are located between the two LTRs. The invention provides Self Inactivating Vector (SIN) feature into the single-LTR based vector system including the development of a conditional-SIN vector, in which the parental U3 region contg. the HIV-1 enhancer/promoter sequence was replaced with a tetracycline-inducible promoter.

IN Kingsman, Alan John; Kingsman, Susan Mary
SO U.S. Pat. Appl. Publ., 21 pp., Cont.-in-part of U.S. Ser. No. 254832.
CODEN: USXXCO
TI Lentiviral LTR-deleted vector for gene therapy targeted to neurons
AB A vector capable of transducing non-dividing and/or slowly dividing cells is provided, wherein the vector is a lentiviral LTR-deleted vector. The invention overcomes the limit of regular HIV-based retroviral vectors, which relies on Tat activation through binding to promoter and TAR within HIV U3 region, by replacing HIV U3 region with murine lentiviral virus MLV U3 region or other desirable promoter (and enhancer) regions. Also provided is a method for producing a protein of interest in a non-dividing or slowly dividing cell by transducing the cell with a lentiviral LTR-deleted vector and expressing the protein of interest in the cell. In addn., target cells contg. the lentiviral LTR-deleted vector are provided. The invention is exemplified by constructing lentiviral LTR-deleted vectors expressing a fusion protein contg. tyrosine hydroxylase (TH) and DOPA decarboxylase (DD) in either TH-DD or DD-TH order, which may be useful for treating Parkinson's disease. The chimeric gene was constructed from cDNAs isolated from a human brain substantia nigra cDNA library with a linker sequence encoding (Gly4-Ser)3. When inserted into a mammalian expression vector (pC1-neo) and used to transiently transfect 293T cells, the fusion gene expresses a fusion protein with dual enzymic activities. Retroviral vectors expressing the TH-DD gene in a single transcription unit configuration are either under control of MLV LTR U3 region or HIV1 LTR U3 promoter.

AU Olson P; Nelson S; Dornburg R
SO Journal of virology, (1994 Nov) 68 (11) 7060-6.
Journal code: 0113724. ISSN: 0022-538X.

TI Improved self-inactivating retroviral vectors derived from spleen necrosis virus.

AB Self-inactivating (SIN) retroviral vectors contain a deletion spanning most of the right long terminal repeat's (LTR's) U3 region. Reverse transcription copies this deletion to both LTRs. As a result, there is no transcription from the 5' LTR, preventing further replication. Many previously developed SIN vectors, however, had reduced titers or were genetically unstable. Earlier, we reported that certain SIN vectors derived from spleen necrosis virus (SNV) experienced reconstitution of the U3-deleted LTR at high frequencies. This reconstitution occurred on the DNA level and appeared to be dependent on defined vector sequences. To study this phenomenon in more detail, we developed an almost completely U3-free retroviral vector. The promoter and enhancer of the left LTR were replaced with those of the cytomegalovirus immediate-early genes. This promoter swap did not impair the level of transcription or alter its start site. Our data indicate that SNV contains a strong initiator which resembles that of human immunodeficiency virus. We show that the vectors replicate with efficiencies similar to those of vectors possessing two wild-type LTRs. U3-deleted vectors carrying the hygromycin B phosphotransferase gene did not observably undergo LTR reconstitution, even when replicated in helper cells containing SNV-LTR sequences. However, vectors carrying the neomycin resistance gene did undergo LTR reconstitution with the use of homologous helper cell LTR sequences as template. This supports our earlier finding that sequences within the neomycin resistance gene can trigger recombination.

AU Hofmann A; Nolan G P; Blau H M

SO Proceedings of the National Academy of Sciences of the United States of America, (1996 May 28) 93 (11) 5185-90.
Journal code: 7505876. ISSN: 0027-8424.

TI Rapid retroviral delivery of tetracycline-inducible genes in a single autoregulatory cassette.

AB We describe a single autoregulatory cassette that allows reversible induction of transgene expression in response to tetracycline (tet). This cassette contains all of the necessary components previously described by others on two separate plasmids that are introduced sequentially over a period of months [Gossen, M. & Bujard, H. (1992) Proc. Natl. Acad. Sci. USA 89, 5547-5551]. The cassette is introduced using a retrovirus, allowing transfer into cell types that are difficult to transfect. Thus, populations of thousands of cells, rather than a few clones, can be isolated and characterized within weeks. To avoid potential interference of the strong retroviral long terminal repeat enhancer and promoter elements with the function of the tet-regulated cytomegalovirus minimal promoter, the vector is self-inactivating, eliminating transcription from the long terminal repeat after infection of target cells. Tandem tet operator sequences and the cytomegalovirus minimal promoter drive expression of a bicistronic mRNA, leading to transcription of the gene of interest (lacZ) and the internal ribosome entry site controlled transactivator (Tet repressor-VP16 fusion protein). In the absence of tet, there is a progressive increase in transactivator by means of an autoregulatory loop, whereas in the presence of tet, gene expression is prevented. Northern blot, biochemical, and single cell analyses have all shown that the construct yields low basal levels of gene expression and induction of one to two orders of magnitude. Thus, the current cassette of the retroviral construct (SIN-RetroTet vector) allows rapid delivery of inducible genes and should have broad applications to cultured cells, transgenic animals, and gene therapy.

AU Hwang, Jung-Joo; Li, Ling; Anderson, W. French

SO Journal of Virology (1997), 71(9), 7128-7131
CODEN: JOVIAM; ISSN: 0022-538X

TI A conditional self-inactivating retrovirus vector that uses a tetracycline-responsive expression system

AB The authors developed a novel conditional self-inactivating (C-SIN) vector, TL-SN, by replacement of the enhancer-promoter of the 3' long terminal repeat of Moloney murine leukemia virus with a synthetic tetracycline operator-cytomegalovirus promoter (tetP) from the tetracycline-responsive expression system (TRES). The other component of the TRES, a chimeric transactivator (tTA), was stably incorporated into

PA317 amphotropic packaging cells, thus generating the packaging cell line PA317-tTA. C-SIN amphotropic G418-resistant virus particles were generated with a titer of 2 .times. 105 CFU/mL within 2 days of transinfection of PA317-tTA cells with TL-SN ecotropic virus particles. This titer was approx. 2 log units higher than that obtained by transinfection of parental PA317 cells and was due to the high level of viral transcripts originating from the tetP promoter at the 5' end of the transduced vector in the presence of tTA. This C-SIN vector has the potential for use in human gene therapy since it incorporates the advantages of previous SIN vectors in having weak tetP promoter activity (in the absence of tTA in target cells) while at the same time achieving high viral titers with PA317-tTA packaging cells.

AU Jaggar R T; Chan H Y; Harris A L; Bicknell R

SO Human gene therapy, (1997 Dec 10) 8 (18) 2239-47.
Journal code: 9008950. ISSN: 1043-0342.

TI Endothelial cell-specific expression of tumor necrosis factor-alpha from the KDR or E-selectin promoters following retroviral delivery.

AB The tumor vasculature offers a target for anti-cancer gene therapy which has the advantages both of good accessibility to systemically delivered therapy and comparative homogeneity across solid tumor types. We aimed to develop retroviruses carrying endothelial-specific promoters for the delivery of genes to proliferating endothelial cells in vitro and to tumor endothelial cells in vivo. This paper reports the generation of such retroviral vectors and the level of expression of murine tumor necrosis factor-alpha (mTNF-alpha) cDNA following infection into endothelial cells and stromal fibroblasts. Retroviral vectors carrying mTNF-alpha have been generated whose expression is controlled either by the retroviral long terminal repeat or by 5' proximal promoter sequences from the endothelial-specific kinase insert domain receptor (KDR)/VEGF receptor and E-Selectin promoters within the context of a self-inactivating (SIN) vector backbone. Both KDR and E-Selectin have been shown to be upregulated on tumor endothelium. A putative polyadenylation sequence AAATAAA within the E-Selectin promoter was mutated to permit faithful transmission of retroviral vectors carrying this promoter. We demonstrate a 10- to 11-fold increase in mTNF-alpha expression from promoter elements within sEND endothelioma cells as compared to NIH-3T3 fibroblasts. Suggestions for further improvements in vector design are discussed.